Application No. 10/018,233
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TC Art Unit: 1753
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AMENDMENT TO THE CLAIMS

1. (Currently Amended) A method of screening a complex material for an unidentified candidate ligand that binds to a pre-selected target, comprising the steps of:

(a) providing a mixture of the complex material and a predetermined concentration of the target, resulting in a target/sample mixture, and separately providing a predetermined concentration of a known, detectable, competitive ligand that binds to the target;

(b) sequentially injecting into a capillary of a capillary electrophoresis instrument having a detector, a first plug of analyte and a second plug of analyte, wherein the first and second plugs of analyte comprise a combination selected from the group consisting of:

(i) a combination of the first plug of analyte being of the target/sample mixture and the second plug of analyte being of the competitive ligand; and

(ii) a combination of the first plug of analyte being of the competitive ligand and the second plug of analyte being of the target/sample mixture;

(c) subjecting the first and second plugs to capillary electrophoresis under conditions optimized for detecting at least one member selected from the group consisting of unbound competitive ligand and a complex of the competitive ligand bound to the target, and optimized so that detected analytes from the second plug migrate faster than detected analytes from the first plug toward the detector and pass through the first plug during capillary electrophoresis, prior to the detected analytes reaching the detector;

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(d) tracking the competitive ligand at the detector to generate a capillary electrophoretic migration pattern; and

(e) determining whether the migration pattern from step (d) differs from a reference standard, thereby indicating the presence of a candidate ligand in the complex material.

2. (Currently Amended) The method of claim 1, wherein the reference standard comprises a reference migration pattern resulting from: (a) capillary electrophoresis of sequential injections of a plug of the predetermined concentration of target without the complex material and a plug of the competitive ligand, using a same order of injection and same capillary electrophoresis conditions as used in claim 1; and (b) tracking the competitive ligand at the detector.

3. (Original) The method of claim 1, wherein the first plug is of the target/sample mixture and the second plug is of the competitive ligand.

4. (Original) The method of claim 3, wherein the competitive ligand has a higher capillary electrophoretic mobility than the target in a direction of the detector.

5. (Original) The method of claim 1, wherein the first plug is of the competitive ligand and the second plug is of the target/sample mixture.

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6. (Original) The method of claim 5, wherein the target has a higher capillary electrophoretic mobility than the competitive ligand in a direction of the detector.

7. (Original) The method of claim 1, 2, 3, or 5, wherein the migration pattern comprises at least one member from the group consisting of a peak representing unbound competitive ligand and a peak representing a complex of the competitive ligand bound to the target.

8. (Original) The method of claim 1, 3, or 5, wherein the target comprises a member selected from the group consisting of enzymes, receptors, proteins, polypeptides, nucleic acids, polynucleotides, carbohydrates, and chemically, enzymatically, or recombinantly modified forms thereof, wherein the modified forms have been modified for improved electrophoretic properties.

9. (Currently Amended) The method of claim 1, 3, or 5, wherein the complex material is selected from the group consisting of combinatorial chemical libraries, extracts of terrestrial plants, extracts of marine plants, cells from higher animals including humans, eubacteria, actinomycetes, bacteria, extracts from non-recombinant or recombinant microorganisms, microbial fermentation broths, fungi, protozoa, algae, archaebacteria, worms, insects, marine organisms, sponges, corals, crustaceans, viruses, phages, tissues, organs, blood, soil, sea water, water from a fresh-water body, humus, detritus, manure, mud, and sewage, or partially purified fractions thereof.

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10. (Original) The method of claim 1, 3, or 5, wherein the known, competitive ligand is a member selected from the group consisting of naturally occurring compounds, synthetic compounds, antibodies, proteins, peptides, and oligonucleotides known to bind to the target.

11. (Original) The method of claim 1, 3, or 5, wherein the known, competitive ligand is detectable by a fluorescence detector.

12. (Currently Amended) The method of claim 1, 3, or 5, wherein the competitive ligand has a dissociation constant (K_d) within a range of about 10μM-100μM and an off-rate (K_{off}) of about 1.0 $(s^{-1}) \leq K_{off} \leq 10\ (s^{-1})$.

13. (Original) The method of claim 12, wherein the predetermined concentration of the competitive ligand is at least about 5.0μM.

14. (Original) The method of claim 1, 3, or 5, wherein the known competitive ligand has a dissociation constant K_d within a range of about 10nM-10μM and an off-rate (K_{off}) within a range of about 0.01 (s^{-1})-1.0 (s^{-1}).

15. (Original) The method of claim 1, 3, or 5, wherein the known competitive ligand has a dissociation constant of about $K_d < 10nM$ and an off-rate of about $K_{off} < 0.01\ (s^{-1})$.

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16. (Original) The method of claim 1, 3, or 5, wherein the predetermined target concentration, predetermined competitive ligand concetration, and capillary electrophoresis conditions are pre-selected to produce, absent any other target-binding ligand, a measurable change in the capillary electrophoretic migration pattern.

17. (Original) The method of claim 16, wherein the measurable change comprises a change of at least 10% in peak area of at least one peak selected from the group consisting of a peak representing unbound competitive ligand and a peak representing a complex of the competitive ligand bound to the target.

18. (Original) The method of claim 16, wherein the measurable change comprises a change of at least 50% in peak area of at least one peak selected from the group consisting of a peak representing unbound competitive ligand and a peak representing a complex of the competitive ligand bound to the target.

19. (Original) The method of claim 16, wherein the measurable change comprises a change of at least 75% in peak area of at least one peak selected from the group consisting of a peak representing unbound competitive ligand and a peak representing a complex of the competitive ligand bound to the target.

20. (Original) The method of claim 16, wherein the measurable change comprises a change of at least 10% in peak area of a peak representing unbound competitive ligand and a change of at least

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10% in peak area of a peak representing a complex of the competitive ligand bound to the target.

21. (Original) The method of claim 1, 3, or 5, wherein capillary electrophoresis conditions are optimized to allow detection of a candidate ligand having a dissociation constant (K_d) within a range of about 10μM-100μM and an off-rate (K_{off}) within a range of about 1.0 (s^{-1})-10 (s^{-1}).

22. (Original) The method of claim 1, 3, or 5, wherein capillary electrophoresis conditions are optimized to allow detection of a candidate ligand having a dissociation constant (K_d) within a range of about 10nM-10μM and an off-rate (K_{off}) within a range of about 0.01 (s^{-1})-1.0 (s^{-1}).

23. (Original) The method of claim 1, 3, or 5, wherein capillary electrophoresis conditions are optimized to allow detection of a candidate ligand having a dissociation constant of about $K_d \leq$ 10nM and an off-rate of about $K_{off} \leq 0.01$ (s^{-1}).

24. (Original) The method of claim 1, 3, or 5, further comprising injecting between the first plug and second plug, a plug of capillary electrophoresis running buffer.

25. (Original) The method of claim 1, 3, or 5, wherein capillary electrophoresis is performed using a running buffer having a pH value within a range of about pH3-pH10.

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26. (Original) The method of claim 1, 3, or 5, wherein capillary electrophoresis is performed using a running buffer having a pH value within a range of about pH5-pH8.

27. (Original) The method of claim 1, 3, or 5, in which capillary electrophoresis is performed using a running buffer having a salt concentration within a range of about 0-500 mM.

28. (Original) The method of claim 1, 3, or 5, in which capillary electrophoresis is performed at a temperature within a range of about 0-60°C.

29. (Original) The method of claim 1, 3, or 5, in which capillary electrophoresis is performed at a temperature within a range of about 5-37°C.

30. (Original) The method of claim 1, 3, or 5, in which capillary electrophoresis is performed with a run time within a range of about 0.5-60 minutes.

31. (Original) The method of claim 1, 3, or 5, in which a distance between a capillary electrophoresis start point and the detector is within a range of about 0.5-1000 cm.

32. (Original) The method of claim 1, 3, or 5, in which capillary electrophoresis is performed in a capillary having a length within a range of about 0.5-1000 cm.

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33. (Original) The method of claim 1, 3, or 5, in which capillary electrophoresis is performed in a conduit of a microchip.

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